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Original Article

Impact of acute administration of sodium oxybate on nocturnal sleep polysomnography and on multiple sleep latency test in narcolepsy with cataplexy

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ABSTRACT

Objective: To analyze the acute effects of sodium oxybate (SO) on polysomnographic night-time recordings (PSG) and multiple sleep latency test (MSLT) on patients with narcolepsy with cataplexy (NC).
Methods: Sixteen NC adult patients were recruited, together with 16 normal controls. Two consecutive PSG followed by two MSLT sessions were carried out, before and during the first night of SO assumption, respectively.
Results: The administration of SO was followed by a significant decrease in number of stage shifts and awakenings, wakefulness after sleep onset, and percentage of sleep stage 1. Sleep efficiency and slow wave sleep percentage increased. REM latency decreased significantly from 73 to 12 min. Cyclic alternating pattern (CAP) rate remained unchanged but the percentage of CAP A3 subtypes decreased. The number of CAP A3 subtypes per hour of NREM sleep decreased significantly, whereas that of A1 remained unchanged. The duration of A1 and A3 subtypes was slightly increased. Chin muscle tone was not modified by SO as well as periodic leg movements during sleep, but their periodicity index decreased, becoming similar to that of controls. MSLT sleep latency also significantly improved after SO intake.
Conclusions: The administration of SO in NC patients is followed by immediate important and complex effects on PSG parameters and MSLT, including an evident (over)increase in slow wave sleep, which does not display a physiological microstructure, a moderate decrease in periodic and isolated LMs, possibly mediated by a disinhibited dopaminergic neuronal activity, and an improvement on daytime mean sleep latency at the MSLT.

1. Introduction

Although cataplexy often displays a dramatic aspect and represents the pathognomonic symptom of narcolepsy with cataplexy (NC), the most frequent major complaint reported by patients when they first seek for medical advice, and the one that largely impacts on quality of life [1], is hypersomnolence, namely excessive daytime sleepiness (EDS). Other typical symptoms are sleep paralysis and hypnagogic or hypnopompic hallucinations, but a significant number of NC patients also have trouble sleeping at night [2]. Subjective complaints of poor sleep quality in patients with NC [3] include vivid frightening dreams [4], inability to sleep without awakening, getting up to eat at night [5], early awakenings or unrefreshed feeling upon awakening in the morning [6], sleep paralysis, and hallucinations [7]. Moreover, patients with NC may present restless legs syndrome [8], and several comorbid conditions can be often found with polysomnographic sleep recording (PSG), such as periodic leg movements during sleep (PLMS), increased isolated leg movements (LMs), obstructive sleep apnea, rapid eye movement (REM) sleep behavior disorder (RBD), and other parasomnia [2,9–11].

Although disrupted nocturnal sleep may have a central role in patients' complaints, traditional pharmacological treatment of NC is only symptomatic, principally focusing on daytime symptoms and based on polytherapy with stimulants and antidepressants.

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Accordingly, the primary endpoints of clinical trials of specific drug treatment for narcolepsy are often limited to the analysis of daytime sleepiness, evaluated with subjective scales and/or with objective tests such as the multiple sleep latency test (MSLT) and the maintenance of wakefulness test (MWT), and cataplexy frequency.

Treatment of NC underwent a genuine change of direction in the last decade, with the registration of sodium oxybate (SO), as a monotherapy, impacting on night-time sleep quality, EDS, and cataplexy. Gamma-hydroxybutyrate, in its salt form, SO, has become the most effective drug to treat both EDS and cataplexy in NC; its efficacy has been assessed in a series of studies [12–15]. Beneficial effects of SO on sleep architecture have been carefully reported in NC patients, with a significant increase in slow wave sleep and a decrease in the amount of sleep stage 1 and of awakenings [16,17]. However, the prescription of SO is still limited, with potential increased risks for sleep breathing disorders, parasomnia (sleepwalking, catathrenia [18], enuresis, and sleep-related eating disorder [19]), and enhancement of periodic limb movement disorder and restless legs syndrome (RLS) [20].

Although SO is believed to improve most symptoms of NC, and also quality of life in these patients [21], there are few data on its effect on motor symptoms and polysomnographically recorded sleep microstructure. For this reason, the aim of this open-label observational two-day study was to analyze, in detail, the acute effects of SO in NC patients on several night-time PSG parameters (sleep architecture, NREM sleep microstructure, leg movement activity during sleep, and the amplitude of the chin EMG during REM sleep) and daytime sleepiness using MSLT.

2. Methods

2.1. Subjects

Sixteen NC adult patients (10 males and six females, mean age 40.2 years, SD: 18.20) were recruited by the Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy. All patients met the International Classification of Sleep Disorders criteria for NC [3] including (a) unequivocal cataplexy; (b) persistent daytime sleepiness; (c) at least two sleep onset REM periods (SOREMPs); and (d) mean sleep latency <8 min at MSLT. The presence of human leukocyte antigen (HLA) DQB1*0602 was assessed in all patients and data on CSF hypocretin-1 level (hcrt-1) were collected when available. For all NC patients, exclusion criteria included (a) sleep apnea syndrome (apnea/hypopnea index <10/h in all cases), (b) major mental/neurologic illness, (c) significant history of cognitive difficulties, and (d) current use of neuroleptic agents. A subjective sleepiness assessment was performed at baseline by means of the Epworth Sleepiness Scale (ESS) [22]. Table 1 reports the clinical details of this group of patients. Seven patients were taking modafinil and venlafaxine and one was taking modafinil alone; the remaining eight were drug-free. Modafinil was discontinued in all patients for at least two weeks before entering the study while venlafaxine was kept stable.

We also selected from our polysomnographic recording database 16 normal controls matched for age to NC patients (eight males and eight females; mean age 42.0 years, SD: 18.64). Healthy control subjects had to be in good health in general and the following exclusion criteria were applied: (a) sleep disorder diagnosis; (b) major mental/neurologic illness; (c) significant history of cognitive difficulties; and (d) prior (within one year) or current use of any psychotropic agent.

All patients gave written informed consent, in agreement with the Convention of Helsinki, to the study protocol that was approved by the local ethics committee.

Table 1
Clinical features of narcoleptic patients and results of the MSLT test at baseline and after treatment with GHB.

Patient	Gender	Age (years)	BMI (kg/m^2)	Disease duration (years)	ESS	DQB1*0602	Hcrt (pg/mL)	RLS symptoms	Other nocturnal disorders		VLFX	Baseline MSLT		GHB MSLT	
									Baseline	GHB		Latency (min)	SOREMP (n)	Latency (min)	SOREMP (n)
NC01	F	32	33.5	26	18	Yes	0	RLS		Eating		5.8	5	5	4
NC02	F	40	NA	11	18	Yes	NA					7.2	4	4.5	4
NC03	M	32	22.4	17	16	Yes	16.8					2.4	3	8.4	1
NC04	M	36	21.3	12	14	Yes	30.1					1.9	5	5.8	3
NC05	F	23	24.4	12	11	Yes	0		RBD		Yes	2.7	5	6.2	0
NC06	M	40	31.6	7	16	Yes	14.3				Yes	4.7	2	7.4	0
NC07	M	16	18.8	8	14	Yes	0					0.8	5	2	3
NC08	F	79	35.2	5	22	Yes	NA					2.8	3	7.8	2
NC09	M	49	27.2	20	22	No	NA	RLS				5.2	3	7.2	1
NC10	M	38	31.3	18	12	Yes	NA		Smoking	Smoking		1.2	5	3.8	4
NC11	M	18	30.9	7	15	Yes	NA				Yes	4.6	3	3.6	3
NC12	F	77	35.2	15	21	Yes	NA				Yes	1.5	3	4.2	1
NC13	M	42	25.2	26	15	Yes	NA	RLS		Groaning, eating, confusional arousal	Yes	2.6	3	2.6	1
NC14	F	43	27.7	11	8	Yes	0		RBD	Eating, smoking	Yes	2.4	3	3.2	5
NC15	M	44	27.5	8	21	Yes	NA					2.8	2	3.0	3
NC16	M	63	29.8	28	11	Yes	10.6	RLS		Enuresis	Yes	1.2	1	1.6	2
Median		40	27.7	12	15.5		5.3					2.6	3	4.35	2.5
1st quartile		32	24	8	13		0					1.7	3	3.1	1
3rd quartile		46.5	31.6	19	19.5		15.5					4.6	5	6.7	3.5

Abbreviations: MSLT, multiple sleep latency test; GHB, gamma-hydroxybutyrate; BMI, body mass index; ESS, Epworth Sleepiness Scale; Hcrt, hypocretin; RLS, restless legs syndrome; VLFX, venlafaxine; SOREMP, sleep onset REM (rapid eye movement) period; RBD, REM sleep behavior disorder; NA, not available.

2.2. Patient study protocol

In all patients included in this study, the withdrawal of modafinil together with the administration of SO was started on the basis of a clinical decision only. The remaining therapy (venlafaxine), when pre-existent, was left unchanged for the whole duration of the study. SO (4.5 g/night, divided into two equal doses) was taken at bedtime, while in bed, and again 2.5–4 h later. Patients had dinner ≥2 h before the first SO intake.

Two consecutive nocturnal polysomnographic studies, each followed by MSLT, were carried out, at baseline, before the introduction of SO, and during the first night of consumption of this substance.

2.3. Nocturnal polysomnography

Nocturnal video-polysomnographic recordings were carried out in a standard sleep laboratory room. Subjects were not allowed caffeinated beverages the afternoon preceding the recordings and were allowed to sleep in until their spontaneous awakening in the morning. Lights-out time was based on individual habitual bedtime and ranged between 22:30 and 23:30. The following signals were recorded: electroencephalogram (EEG) (at least four channels, including C3 and C4, referred to the contralateral mastoid); electrooculogram (electrodes placed 1 cm above the right outer cantus and 1 cm below the left outer cantus and referred to the left mastoid); electromyogram (EMG) of the submentalis muscle; EMG of the right and left tibialis anterior muscles (bipolar derivations with two electrodes placed 3 cm apart on the belly of the tibialis anterior muscle of each leg; impedance was kept <10 KΩ); and electrocardiogram (CM4 derivation: anode in position V4 and cathode attached to the manubrium of the sternum). Sleep signals were sampled at 200 Hz and stored on hard disk in European data format for further analysis. EMG signals, in particular, were digitally band-pass filtered at 10–100 Hz, with a notch filter at 50 Hz. The sleep respiratory pattern of each patient was monitored using oral and nasal airflow thermistors and/or nasal pressure, thoracic and abdominal respiratory effort strain gauge, and by monitoring oxygen saturation (pulse oximetry).

2.4. Sleep scoring and CAP analysis

Sleep stages were scored following standard criteria on 30 s epochs [23]. Subsequently, each CAP A phase was detected in each recording on the C3/A2 or C4/A1 derivation; the side of this EEG channel should not influence the detection of CAP because CAP components have been shown to map symmetrically over the scalp [24]. All CAP phases during NREM sleep were detected and classified into three subtypes (A1, A2, and A3) according to Terzano et al. [25].

CAP was detected by the sleep analysis software Hypnolab 1.2 (SWS Soft, Italy) which allows computer-assisted detection of CAP A phase subtypes. With this software, detection is performed by means of a human-supervised automatic approach controlled by the scorer. The performance of this system has been evaluated and validated [26], but for this study the scorer visually edited the detections proposed by the automatic analysis, before the computation of the various CAP parameters which were automatically generated by the same software and used for statistical analysis. In order to reduce the effects of the inter-rater variability expected for this type of analysis [26], all recordings were scored by one of the authors of this study (D.A.).

2.5. Scoring rules for the cyclic alternating pattern

CAP is organized in sequences of two or more CAP cycles. Each CAP cycle consists of phase A and phase B, each lasting between 2 and 60 s. All CAP sequences begin with phase A and end with phase B.

On the basis of the information derived from EEG activities, muscle tone, and neurovegetative responses, a three-stage hierarchy of arousal strength can be identified as follows.

2.5.1. Subtype A1

These are A phases in which EEG synchrony is the predominant activity. In A1 phases the following activities are included: intermittent alpha rhythm and vertex sharp waves during stage 1, and sequences of k-complexes or delta bursts in other NREM stages. If present, EEG desynchronization occupies <20% of the entire phase A. Subtype A1 is generally associated with mild autonomic and somatomotor activity.

2.5.2. Subtype A2

These are A phases which contain a mixture of slow and rapid EEG activities. In A2 phases the following activities are included: K-alpha, arousal preceded by slow wave synchronization and polyphasic bursts with >20% but <50% of EEG desynchronization. Subtype A2 is linked with a moderate increase of muscle tone and/or cardiorespiratory rate.

2.5.3. Subtype A3

These are A phases with predominant EEG desynchronization, including arousals, transient activation phases or polyphasic bursts with >50% of EEG desynchronization. Subtypes A3 are coupled with a remarkable enhancement of muscle tone and/or cardiorespiratory rate.

CAP rate is generally calculated as the percentage of NREM sleep or of each NREM sleep stage occupied by CAP sequences; for a complete set of rules and examples for scoring CAP, refer to Terzano et al. [25].

2.6. Analysis of leg movements during sleep

Leg movements during sleep were detected following the WASM-IRLSSG criteria [27]. The PLMS index was calculated as the number of LMs included in a series of four or more, separated by >5 and <90 s, per hour of sleep.

Similar to our previous studies on PLMS intervals, a distribution histogram of all inter-LM intervals was obtained [11,28] and, subsequently, the number of intervals included in sequences of at least three, all 10–90 s long, was divided by the total number of intervals: this ratio is referred to as the periodicity index, which can vary between 0 (absence of periodicity, with none of the intervals having a length between 10 and 90 s) to 1 (complete periodicity, with all intervals having a length between 10 and 90 s) [28]. Periodicity index is independent of the absolute number of LMs recorded and has been calculated for all the subjects included in this study.

2.7. Quantification of the submentalis muscle EMG amplitude

The submentalis muscle EMG signal was analyzed following a previously reported method [10,29,30] and the REM sleep atonia index was computed accordingly. Mathematically, this index can vary from 0, which means complete absence of EMG atonia, to 1, or continuous stable EMG atonia during REM sleep. Subsequently, chin muscle activations were counted and their number per hour of REM sleep was calculated.

2.8. Statistical data analysis

The comparison between findings obtained in normal controls and NC patients at baseline was carried out using the non-parametric

Table 2
Sleep architecture parameters in controls and in narcoleptic patients at baseline and after treatment with GHB.

	1. Controls		2. NC baseline		3. NC GHB		*P*-value Mann–Whitney 2 vs 1	*P*-value Wilcoxon 2 vs 3
	Median	1st–3rd quartile	Median	1st–3rd quartile	Median	1st–3rd quartile		
Time in bed (min)	430.5	399.5–484.5	503.8	424.3–518.0	427.0	404.8–494.0	NS	0.049
Sleep period time (min)	408.3	382.3–471.8	489.8	421.8–507.8	421.5	393.8–483.5	NS	0.044
Total sleep time (min)	394.3	354.0–419.5	382.5	300.5–410.3	367.5	330.8–412.8	NS	NS
Sleep latency (min)	10.5	4.8–18.3	3.0	2.3–5.0	2.0	1.5–3.3	0.002*	NS
REM sleep latency (min)	64.5	55.0–78.3	73.5	20.0–114.5	12.3	3.3–50.3	NS	0.017
Stage shifts (no./h)	8.7	7.5–10.9	15.3	11.1–19.5	9.3	6.5–14.7	0.0007*	0.0004*
Awakenings (no./h)	1.9	0.9–2.8	11.7	8.3–14.2	5.3	3.0–7.9	0.000003*	0.0004*
Sleep efficiency (%)	90.9	84.9–95.3	76.5	70.0–84.2	85.5	76.6–92.2	0.0037*	0.026
Wakefulness after sleep onset (%)	3.7	1.1–9.7	22.8	14.7–29.6	12.1	6.5–19.5	0.0005*	0.008
Sleep stage 1 (%)	1.2	0.5–3.2	18.4	11.7–24.0	5.7	2.6–12.4	0.000012*	0.0008*
Sleep stage 2 (%)	46.2	42.4–53.5	31.1	24.1–37.8	27.9	20.7–38.2	0.001*	NS
Slow wave sleep (%)	21.0	17.5–26.7	9.8	3.9–14.7	37.5	22.4–53.7	0.003*	0.0006*
REM sleep (%)	21.2	16.5–25.9	14.3	9.5–18.0	15.2	8.7–19.4	0.007	NS

The percentages of wakefulness after sleep onset and sleep stages are referred to sleep period time.
Abbreviation: NS, not significant.
* Significant after Bonferroni correction.

Mann–Whitney test for independent data sets. For the comparison between the results obtained in NC patients at baseline and at follow-up after SO therapy, the non-parametric Wilcoxon test for paired data sets was used. The level of significance was set at $P < 0.05$; additionally, a more restrictive *P*-level was also used, after Bonferroni correction, in order to take into account the effect of multiple comparisons. Fisher's exact test was also used for the comparison of the frequency of detection of behavioral events during the two recording nights. The data analysis software system Statistica (StatSoft, Inc. 2004, version 6. http://www.statsoft.com) was used for statistical analysis.

3. Results

3.1. Clinical and MSLT findings

Table 1 reports the individual clinical features of the patients enrolled and the findings in the total group expressed as median and interquartile range. The HLA DQB1*0602 allele was found in 15 out of the 16 patients.

Four patients had RLS symptoms and two patients had full-blown RBD episodes only during the baseline video-PSG recording (not significant by Fisher's exact test when compared with the second night recording). One patient had repeated (five) episodes of nocturnal cigarette smoking during periods of wakefulness, at baseline which persisted under SO treatment (three episodes); in another patient, nocturnal smoking occurred only in the second recording night. Similarly, nocturnal awake eating episodes occurred only under SO treatment in three patients (two of whom with RLS and the other with RBD episodes during the first night; all these patients had a personal history of nocturnal eating/smoking). Nocturnal groaning and confusional arousal were observed in one patient with RLS symptoms and nocturnal eating. Finally, enuresis occurred in one patient with RLS symptoms during the second night recording, under SO treatment. Altogether, nocturnal eating, groaning, confusional arousal, and enuresis were observed in four patients under SO treatment and in none at baseline ($P = 0.05$ with Fisher's exact test).

MSLT latency was 2.6 min (1.7–4.6) at baseline and 4.3 min (3.1–6.7) after the first SO treatment night (Wilcoxon test, $P = 0.017$) whereas the number of SOREMPs was 3 (3–5) at baseline and 2.5 (1–3.5) after the second night (Wilcoxon test, $P = 0.02$).

No changes in the nocturnal respiratory pattern were found and all patients continued to have apnea/hypopnea index <10/h after treatment.

3.2. Sleep architecture

Table 2 shows the sleep architecture parameters in controls and in narcoleptic patients at baseline and under SO. The comparison of the results obtained at baseline in NC patients and those obtained in normal controls yielded several significant differences, as expected, with NC patients showing increased number of stage shifts and of awakenings, percentage of wakefulness after sleep onset, and percentage of sleep stage 1; sleep latency, sleep efficiency and percentage of sleep stage 2, slow wave sleep, and REM sleep were decreased.

The administration of SO was followed by a significant decrease in number of stage shifts and awakenings, wakefulness after sleep onset, and percentage of sleep stage 1; on the contrary, sleep efficiency and percentage of slow wave sleep increased.

3.3. Cyclic alternating pattern

The results of the CAP analysis are reported in Table 3. At baseline, NC patients showed a decrease in CAP rate during slow wave sleep, due to the significant reduction in slow-wave-containing CAP A1 subtypes (percentage and number/h), and an increase in fast-wave-containing CAP A2 and A3 subtypes (percentage). The duration of all CAP A subtypes was reduced in NC patients; conversely, the duration of the B phase of CAP (return to baseline amplitude) was increased. Finally, the number and duration of CAP sequences was significantly decreased.

After SO therapy, CAP rate remained unchanged but the percentage of CAP A1 subtypes increased whereas that of A3 subtypes decreased. The number of CAP A3 subtypes per hour of NREM sleep decreased significantly. The duration of A1 and A3 subtypes was slightly increased.

Fig. 1 shows the number of CAP intervals in controls and in narcoleptic patients at baseline and under SO treatment, during sleep stages 1 + 2, and during slow wave sleep. Significant differences are evident between controls and NC patients at baseline, especially for the evident reduction of the peak between 20 and 50 s in patients during slow wave sleep, which remains unmodified under SO treatment.

3.4. Leg movement activity during sleep

Almost all LM activity parameters considered in this study were higher in NC patients than in controls; PLMS and isolated LM indexes were increased in both REM and NREM sleep, as well as the number

Table 3
Cyclic alternating pattern parameters in controls and in narcoleptic patients at baseline and after treatment with GHB.

	1. Controls		2. NC baseline		3. NC GHB		*P*-value Mann–Whitney 2 vs 1	*P*-value Wilcoxon 2 vs 3
	Median	1st–3rd quartile	Median	1st–3rd quartile	Median	1st–3rd quartile		
Total CAP rate (%)	36.7	31.7–40.4	17.2	11.4–22.7	17.0	10.3–28.2	0.0044	NS
in sleep stage S1	4.4	0.0–39.2	10.0	2.3–12.5	10.8	5.4–13.5	NS	NS
in sleep stage S2	22.6	16.9–30.6	13.6	9.3–25.1	15.8	10.4–27.2	0.05	NS
in slow wave sleep	64.2	48.0–81.7	27.8	13.0–44.1	21.8	14.3–26.9	0.005	NS
A1 subtypes (%)	80.8	76.2–84.1	48.0	19.6–57.1	64.3	57.1–78.5	0.000077*	0.001*
A2 subtypes (%)	10.0	6.2–13.5	11.2	8.5–20.3	10.0	7.4–15.7	NS	NS
A3 subtypes (%)	10.5	7.9–12.3	41.4	25.3–64.9	20.8	13.0–33.3	0.000065*	0.0038
A1 mean duration (s)	8.0	7.1–8.7	5.3	5.1–5.9	5.5	5.3–7.0	0.000006*	0.04
A2 mean duration (s)	10.7	8.7–12.7	7.9	6.7–10.3	8.8	7.4–11.5	0.028	NS
A3 mean duration (s)	14.4	13.1–15.8	11.1	8.3–12.9	12.5	9.5–15.6	0.0028*	0.011
A1 index (no./h)	38.7	34.5–42.9	12.6	3.8–22.2	12.6	8.4–23.8	0.000055*	NS
A2 index (no./h)	3.9	2.5–6.5	2.2	1.8–3.8	2.1	1.6–3.7	NS	NS
A3 index (no./h)	2.8	2.0–4.7	8.0	3.0–14.2	3.2	1.3–7.5	0.030	0.02
B phase mean duration (s)	23.1	20.4–24.4	25.3	23.9–30.0	26.3	24.2–28.2	0.001*	NS
CAP cycle mean duration (s)	32.1	30.4–32.8	34.6	32.8–37.8	33.9	32.6–34.5	0.006	NS
CAP sequence mean duration (s)	227.8	177.4–268.1	145.5	117.3–163.6	130.1	116.1–178.4	0.0034	NS
No. of CAP sequences	30.0	22.5–37.5	22.0	15.0–30.0	25.0	18.0–35.0	0.046	NS

Abbreviations: GHB, gamma-hydroxybutyrate; NC, narcolepsy with cataplexy; CAP, cyclic alternating pattern; NS, not significant.
* Significant after Bonferroni correction.

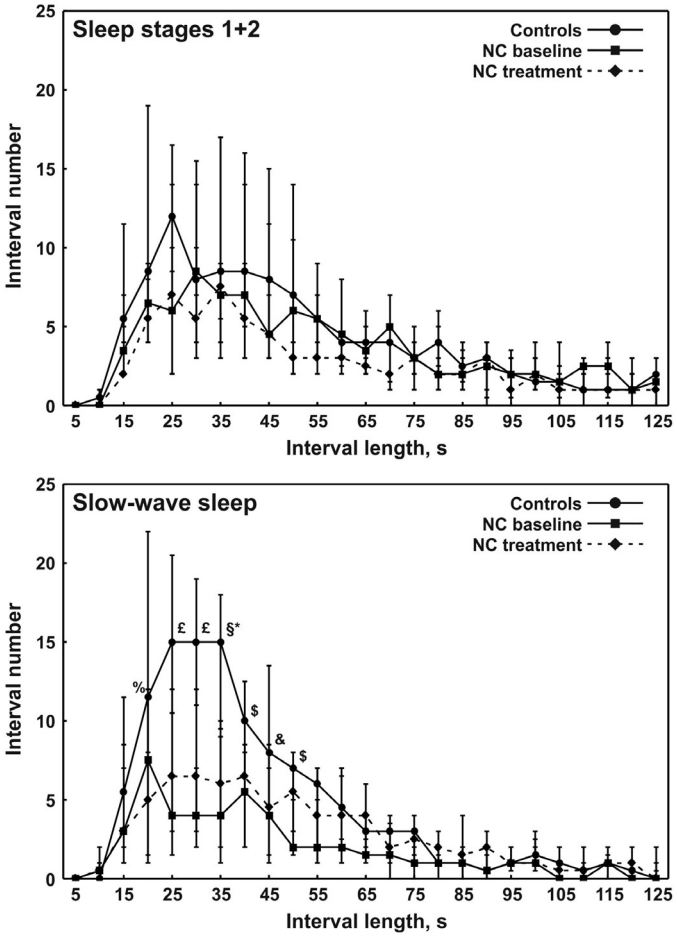

Fig. 1. Distribution histograms of the number of cyclic alternating pattern intervals in controls and in narcoleptic patients at baseline and under gamma-hydroxybutyrate treatment, during sleep stages 1 + 2 (upper panel) and slow wave sleep (lower panel). Data are shown as median and interquartile range (whiskers). The results of the statistical comparison between controls and NC patients at baseline are reported (Mann–Whitney test: $^{\%}P < 0.05$, $^{\&}P < 0.04$, $^{\$}P < 0.025$, $^{£}P < 0.003$, $^{\S}P < 0.0009$). *Significant after Bonferroni correction.

of PLMS sequences and their duration. Finally, the periodicity index was slightly higher than that of normal controls but failed to reach statistical significance (Table 4).

Under therapy with SO, there was a general moderate decrease in total sleep LM activity which was significant during NREM sleep. The number of PLMS sequences was significantly decreased after SO, as was the periodicity index.

Fig. 2 (upper panel) shows the leg intermovement intervals in controls and in NC patients. Both groups show a somewhat similar distribution of intermovement intervals, with a main peak on the extreme left of the graphs, followed by a gradually decreasing trend with increasing interval length. However, the graph of NC patients is clearly higher than that of controls at least up to intervals as long as 90 s with the difference reaching statistical significance most often for intervals of 10–50 s. NC patients show only a very dubious and small peak in the "periodic" range (10–40 s) [11,28].

Fig. 2 (lower panel) shows the leg intermovement intervals in NC patients at baseline and under SO therapy. A small general decrease of the graph is evident, extending approximately between 2 and 50 s but reaching statistical significance only at very few points.

In Fig. 3 (upper panel) the distribution of the number of PLMS and isolated LMs per hour of night in controls and in NC patients are compared. Both PLMS and isolated LM were always higher in NC patients, with statistical significance reached only in some instances. PLMS in NC tended to show a bell-shaped distribution [11].

Fig. 3 (lower panel) shows the changes observed in these graphs after SO administration. PLMS tended to be lower throughout the entire night, after SO. The effects on the night distribution of isolated LM were less evident but reached statistical significance for the sixth and eighth recording hours.

3.5. Chin tone during REM sleep

Median REM sleep atonia index at baseline in NC patients was 0.895 (interquartile range, 0.591–0.920) vs 0.966 (0.902–0.974) in normal controls ($P < 0.009$, Mann–Whitney test); after SO administration, atonia index remained substantially unchanged (0.892; 0.799–0.955). Similarly, the median number of chin muscle activations at baseline in NC patients was 91.4 (59.3–256.8) vs 30.4 (22.4–83.4) in normal controls ($P < 0.01$, Mann–Whitney test); after SO administration, it remained substantially unchanged (81.0; 54.2–177.0).

Table 4
Leg movement parameters in controls and in narcoleptic patients at baseline and after treatment with GHB.

	1. Controls		2. Narco baseline		3. Narco GHB		*P*-value Mann–Whitney 2 vs 1	*P*-value Wilcoxon 2 vs 3
	Median	1st–3rd quartile	Median	1st–3rd quartile	Median	1st–3rd quartile		
Total sleep								
Total LM, index	8.6	4.4–11.9	39.3	15.5–50.5	27.1	9.7–37.7	0.00034*	0.015*
PLMS, index	2.7	0.2–5.3	25.2	4.7–41.2	18.3	2.0–26.3	0.0023*	NS
Isolated LM, index	4.6	3.9–6.7	10.8	8.4–14.3	8.4	7.4–10.5	0.00003*	0.0016*
NREM sleep								
Total LM, index	7.4	2.6–11.7	40.7	15.6–54.7	25.1	7.8–36.9	0.00034*	0.044
PLMS, index	2.2	0.0–5.0	25.2	4.9–47.3	17.3	2.5–25.9	0.0016*	NS
Isolated LM, index	3.9	2.5–6.4	9.7	6.5–12.7	7.4	5.3–9.7	0.00012*	0.0045
REM sleep								
Total LM, index	9.4	5.9–13.5	31.3	14.8–41.4	23.2	13.5–34.6	0.016	NS
PLMS, index	0.2	0.0–2.0	5.9	1.8–24.7	4.5	0.0–16.9	0.018	NS
Isolated LM, index	8.2	4.6–11.3	17.2	10.6–19.8	12.7	7.9–19.8	0.0088	NS
PLMS sequence (number)	3.0	0.0–5.5	16.0	5.0–26.0	11.0	2.0–14.0	0.0014*	0.032
PLMS sequence duration (s)	0.0	0.0–0.0	34.5	9.2–105.3	10.1	0.0–62.2	0.012	NS
PLMS duration (REM) (s)	1.6	0.0–2.6	2.5	2.2–3.2	2.4	0.9–3.0	NS	NS
PLMS duration (NREM) (s)	2.3	1.4–3.4	2.6	2.4–3.7	2.7	2.4–3.5	NS	NS
Isolated LM duration (REM) (s)	2.4	1.2–3.3	2.6	2.1–3.6	2.3	1.8–2.9	NS	NS
Isolated LM duration (NREM) (s)	2.5	1.8–2.8	2.7	2.3–3.2	2.5	2.3–3.0	NS	NS
Periodicity index	0.167	0.000–0.570	0.378	0.244–0.566	0.237	0.172–0.331	NS	0.02

Abbreviations: GHB, gamma-hydroxybutyrate; LM, leg movements; PLMS, periodic leg movements during sleep; NS, not significant; REM, rapid eye movement; NREM non-rapid eye movement.
* Significant after Bonferroni correction.

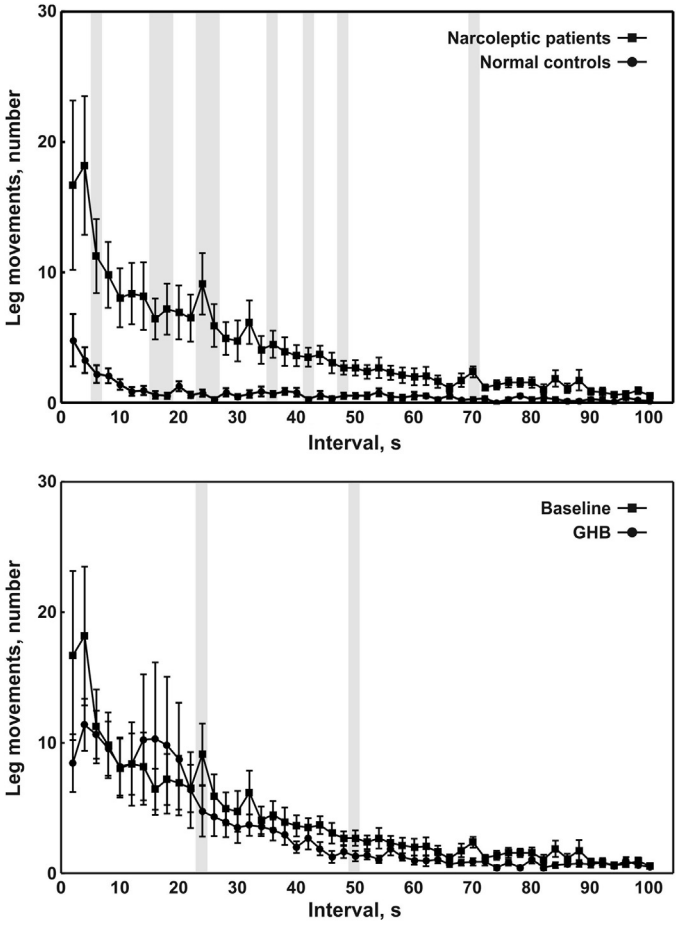

Fig. 2. Distribution histograms of leg intermovement intervals in controls and in narcoleptic patients (upper panel) at baseline and after gamma-hydroxybutyrate therapy (lower panel). The grey shaded areas indicate significant differences (with Bonferroni correction) between the histograms (upper panel, Mann–Whitney; lower panel, Wilcoxon test; $P < 0.01$).

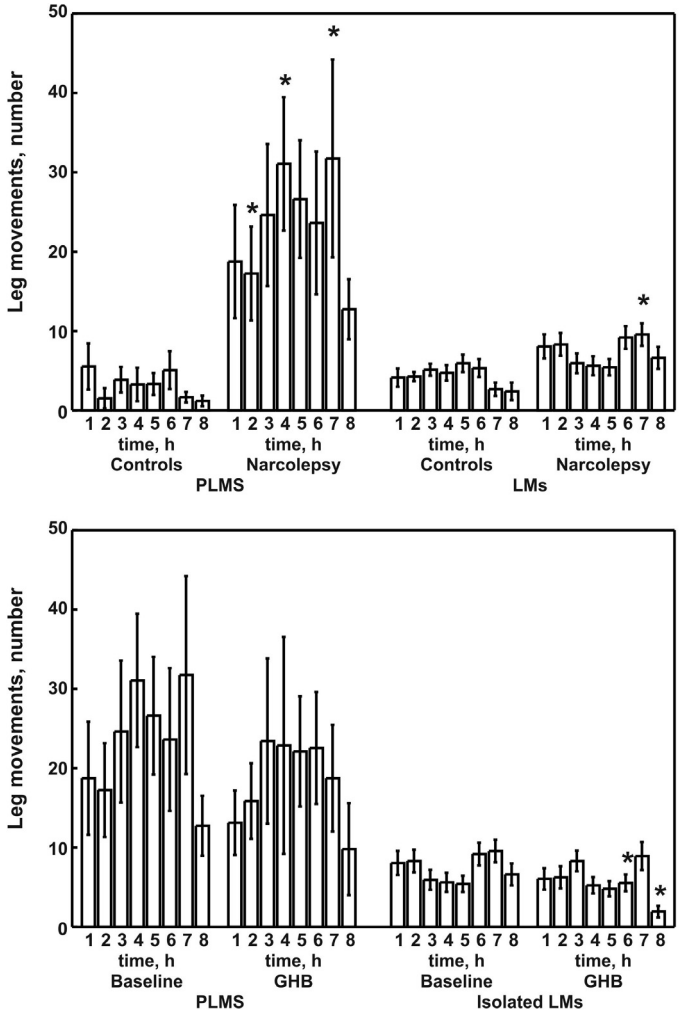

Fig. 3. Number of periodic leg movements during sleep (PLMS) and isolated leg movements (LMs) per hour of night in controls and in narcoleptic patients (upper panel) at baseline and after gamma-hydroxybutyrate (GHB) therapy (lower panel). The asterisks indicate significant differences (with Bonferroni correction) between the histograms (upper panel, Mann–Whitney; lower panel, Wilcoxon test; $P < 0.01$).

4. Discussion

The present study shows that the acute administration of SO at the dosage of 4.5 g per night is well tolerated. Although some of the known side-effects may appear, these do not alter the respiratory function during sleep, and do not substantially impact on sleep architecture and microstructure, also exerting an immediate positive effect in ameliorating EDS. Mean sleep latency at MSLT increased, although remaining <8 min in almost all patients. Even if only few controlled trials are available on the efficacy and safety of SO, this substance is believed to be well tolerated in patients with NC and most adverse events are mild to moderate in severity [31]. Most of these studies reported only a relatively short follow-up (2–12 weeks) during which a significant reduction in cataplexy attacks and EDS could be observed, with an improvement in EDS appearing after eight weeks of treatment [14,32]. Our study demonstrates an immediate improvement in objective EDS. In one report, four NC patients were treated with SO and followed for approximately two years; cataplexy and EDS seemed to have improved during the entire follow-up period [33].

Patients receiving SO have been reported to present some adverse events, including nausea, vomiting, and dizziness [31]. Also a statistically non-significant trend has been reported for increased enuresis vs placebo and for significantly more frequent urinary incontinence [34]. One of our patients presented enuresis during the first night of treatment, a common problem in studies on the effects of SO, but a known transient side-effect in clinical experience [31]. The same applies to the apparent high frequency of occurrence of events, possibly correlated to NREM sleep, such as confusional arousals, and other nocturnal behaviors such as eating and smoking that emerged during the treatment night. Groaning is a known and often transient side-effect [16]. On the contrary, clear RBD episodes were only observed at baseline in two patients; this positive effect of SO on RBD has been reported anecdotally [35], but in our study quantitative chin tone parameters remained unchanged, suggesting that mechanisms responsible for REM sleep without atonia are probably different from those triggering RBD; this is in agreement with recent data on idiopathic RBD showing that, despite the good response of RBD episodes to clonazepam, no effect was detected on REM sleep without atonia [36,37].

Several polysomnographic characteristics have been reported in drug-free patients with narcolepsy, including increased awakenings after sleep onset, sleep stage 1 and number stage shifts, and reduced slow-wave sleep [2,38]. There is convergent evidence that SO causes a significant increase in slow wave sleep and delta EEG power [16,17,33,39–41]; also, increased total sleep time and decreased sleep stage 1, wake after sleep onset, and night-time awakenings have been reported [17,39,41]. Our results are in full agreement with the previous evidence with a significant reduction of stage shifts, number of awakenings, and percentage of sleep stage 1, and an increase in slow wave sleep observed during the first treatment night with SO. This indicates that the known effects of SO on sleep architecture of NC at short term (weeks) are already detectable during the first treatment night, and, perhaps also indicates that the effects observed on the other variables included in this study might be considered as representative of the short-term effects of SO. Interestingly, REM latency was significantly decreased in this study; it is known that first doses of SO can trigger disagreeable sleep paralysis and this finding may be correlated with this clinical observation even if it was not reported by our patients.

Moreover, it is interesting to note that slow wave sleep under SO in NC patients exceeded the amount of this stage observed in normal controls (Mann–Whitney test, $P < 0.013$); thus, SO seems to exert an overcorrection of the decreased basal slow wave sleep in NC.

Slow waves are the main component of the CAP A1 subtypes [24,25], and a reduced total CAP rate, with a prominent reduction in the number of A1 subtypes/hour has been previously reported in NC [42–44] and fully confirmed in this study at baseline. However, SO was unable to increase the A1 index and only increased their percentage as an effect of the reduction in the number of the arousal-related fast-wave-containing A3 subtypes, which were reduced to values similar to those of normal controls. The total CAP rate remained unchanged. Additionally, analysis of the time structure of CAP (Fig. 1) disclosed that the abnormally low sleep instability of our NC patients was particularly evident during slow wave sleep and that this abnormality persisted under SO treatment. Thus, even if SO is able to augment slow wave sleep, this sleep stage appears to be structurally abnormal and with a persistent reduction of its physiological instability. This finding might lead to some important speculative considerations because of the involvement of the A1 CAP subtypes in sleep-related cognitive processes [45–47], which might be the basis for future assessment of cognitive capabilities in NC patients under SO treatment. In support of this, it has already been shown that SO can impair performance on learning and memory tasks [48–51], perhaps as a consequence of an increased oxidative stress in the brain and neurotoxic damage to the hippocampus [51,52].

Increased leg movement activity during sleep is common in NC patients [11,53] but it has been shown to be periodic when RLS is also present [54]; in this study, only a minority of patients had RLS symptoms and, for this reason, we pooled their data together with those of others and the median periodicity index was found to be somewhat lower than in the previous studies [11,54] without reaching statistical significance when compared with controls. However, significantly higher parameters of leg movement activity during sleep were found in NC patients at baseline, as expected, in both NREM and REM sleep. The time structure of the leg movement activity (intermovement distribution and number of movements/hour of night) remained substantially unchanged. The interpretation of these results is difficult and can only be speculative because the features of the movements found in the present study only match partially with those of the movements known to respond to dopamine agonists [55]. However, the periodicity index tended to be higher than that of controls and the intermovement interval distribution showed significant differences from normal controls almost exclusively in the 16–48 s range; these intervals fall within the range responding to dopamine agonists [55]. Thus, it is possible to hypothesize that at least a portion of the movements recorded in our NC patients might be potentially responsive to dopaminergic agents.

Before concluding, it should be acknowledged that this study had limitations: it was an open trial; the sample size was small; heterogeneous comorbidities (RLS, RBD) were found, a common aspect of NC; we only used the starting low dose of SO; we analyzed only the very first effects of SO during the first administration night; and some patients were also under venlafaxine treatment.

In conclusion, the administration of SO in NC patients is followed by immediate and complex effects as soon as the first treatment night. These effects, resulting in one prompt improvement in EDS, include an evident (over)increase in slow wave sleep without changes in the physiological sleep microstructure (CAP) that is affected in NC. Replication and confirmation of these results in long-term studies are now warranted for a better pharmacological management of NC, together with the analysis of their eventual clinical effects.

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Conflict of interest

Prof. Plazzi has consulted for UCB Pharma and Jazz pharmaceuticals. Dr Ferri has consulted for Merck & Co., Sapio-Life and EB Neuro, and has spoken on behalf of UCB Pharma. Prof. Dauvilliers has consulted for Bioproject, UCB Pharma and Jazz pharmaceuticals. Prof. Bruni has consulted for Sapio Life. Drs Aricò, Pizza, and Vandi have no potential conflicts of interest.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.04.020.

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